REMARKS

In the specification, various paragraphs have been amended to correct minor editorial errors. The amendment on page 22 regarding the ion-exchange column merely reflects the inherent anionic-exchange property of the DEAE column and does not introduce new matter.

Claims 1, 3-22, and 27-35 remain in this application. Claims 2 and 23-26 have been canceled, without prejudice or disclaimer, and applicants expressly reserve the right to file one or more continuing applications directed thereto. Claims 1, 6, 13, and 27 are amended. No new matter is added by the amendments. Claim 1 incorporates the recitations of canceled claim 2 and is amended for clarification purposes as noted below, support being found, for example, on page 14, line 1, and page 17, line 13. Support for the amendments to claims 6 and 27 is found throughout the specification, such as, for example, the last paragraph of Example 3 regarding TPO (page 37, line 49 to page 38, line 3) and the last paragraph of Example 5 regarding FGF-5 (page 41, lines 29-32), indicating that this process works to increase percentages of two very different full-length heterologous polypeptides relative to the total amount of full-length and truncated such polypeptides. Further, claims 6 and 13 are amended for clarification purposes as noted below, support being found at least on page 13, lines 35, 36, and 38, page 20, lines 20, 23, 28, and 31-32, page 21, line 15, page 23, line 36, page 38, line 12, and page 39, lines 33 and 37.

Rejection under 35 U.S.C. § 112, Second Paragraph

Claims 1-22 are rejected under 35 U.S.C. § 112, second paragraph, for two reasons:

1. The vector is stated in claim 1 as comprising RNA rather than DNA. Without acquiescing in the rejection, and to clarify claim 1 but not alter its scope, the word "RNA" in claim 1 is changed to "DNA" as suggested by the Examiner. In view of this amendment, dependent claims 3-5 are free of this rejection. Claim 2 is canceled.

2. In claim 6, the cells are described as comprising "RNA encoding the polypeptide with a non-lambda promoter therefor." The Examiner is of the view that the claim should read that the RNA is "expressed from a gene with a non-lambda promoter." Without acquiescing in the rejection, and to clarify claim 6 but not change its

scope, the expression is changed to "RNA encoding the polypeptide wherein the RNA is expressed from a gene with a non-lambda promoter." Claim 13 is amended to conform to this new language. The remaining rejected claims, which ultimately depend from claim 6, are free from the rejection in view of these amendments to the independent claim.

In view of the above, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-22 under 35 U.S.C. §112, second paragraph.

Rejection under 35 U.S.C. § 102(b) (Hasan and Szybalski, Gene, 56: 145-151 (1987))

Claims 1 and 3 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Hasan. The Examiner's position is that Hasan discloses in Figures 1 and 2 plasmids designated for the expression of proteins in prokaryotic cells comprising either a P*lac* or P*tac* promoter, a nutL site, the N gene, and a gene of interest, which is galK. The vectors are designed to express the protein of interest in *E. coli* cells.

Applicants respectfully traverse this rejection. Anticipation requires that all of the elements and limitations of the claims be found within a single prior art reference. There must be **no difference** between the claimed invention and the reference disclosure as viewed by one of ordinary skill in the art. *Scripps Clinic & Research Fdn. v. Genentech*, 927 F.2d 1565, 1576 (Fed. Cir. 1991) (emphasis added). Absence from a cited reference of any element of a claim negates anticipation of that claim by that reference. *Atlas Powder Co. v. E.I. DuPont de Nemours & Co.*, 224 USPQ 409 (Fed. Cir. 1984).

The only gene of interest being expressed by the vector in Hasan is *galK*. However, the *galK* gene used in Hasan is **bacterial**, and not a heterologous polypeptide as required by instant claims 1 and 3. This is evident from the following trail of references: All of the plasmids in the Hasan paper are derived from the plasmid pNH7a - see the third sentence in the summary of Hasan on page 145. The plasmid pNH7a is described in a paper by Podhajska, Hasan, and Szybalski (1985) cited by Hasan on page 151 (right column). Figure 1 on page 165 of this Podhajska paper (a copy of which is enclosed with this Amendment along

with an Information Disclosure Statement (IDS) citing it) states that plasmid pNH7a is a derivative of the plasmid pKO1, citing a McKenney *et al.* (1981) reference, a copy of which is also enclosed with this Amendment and cited in the enclosed IDS. (The McKenney *et al.* reference is also cited on page 151 (right column) of the Hasan paper.) The McKenney *et al.* reference discusses the origin of the plasmid pKO1 (calling it pKO-1) and why they chose the *E. coli galK* gene contained therein. Among other pages, page 387 of McKenney *et al.* states that the *galK* gene in the plasmid is an *E. coli galK*.

Since this traced nexus to Hasan clearly evidences that the *galK* gene employed by Hasan is bacterial, the rejected claims are not anticipated thereby, because the polypeptide of the rejected claims 1 and 3 is heterologous to the prokaryotic cells.

Further, since claim 1 is now amended to recite the language of claim 2, it is further differentiated from Hasan, since Hasan contains absolutely no teaching regarding GreA or GreB.

Because Hasan does not disclose each and every element of the claimed invention as required by the law of anticipation set forth above, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1 and 3 under 35 U.S.C. §102(b) over Hasan.

Rejection under 35 U.S.C. § 103(a) (Hasan in view of Hsu *et al.*, Proc. Natl. Acad. Sci. USA, 9: 11588-11592 (1995))

Claims 1-3 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hasan in view of Hsu. Hasan is disclosed above. Hsu is cited as disclosing plasmids that express GreA and GreB and as showing that they are functional in increasing the expression of the CAT gene (Figure 3). The Examiner's position is that since the goal of both Hasan and Hsu is to allow for the high-level expression of proteins, one skilled in the art would have been motivated to construct a vector comprising both the nut site and N gene of Hasan and the GreA- or GreB-encoding gene as taught by Hsu.

A finding of obviousness under 35 U.S.C. § 103 requires a determination of the scope and content of the prior art, the differences between the invention and the prior art, the level of ordinary skill in the art, and whether the differences are such that

the claimed subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v. Deere*, 383 U.S. 1 (1966). Once the scope and content of the prior art is determined, the relevant inquiry is whether the prior art as a whole suggests the invention, and whether one of ordinary skill in the art would have had a reasonable expectation that the claimed invention would be successful. *In re Vaeck*, 20 USPQ 2d 1438 (Fed. Cir. 1991). When the references are combined to support a rejection, there must be some teaching in the references themselves that suggests the combination. If an explicit suggestion or teaching is missing from a reference, it cannot be supplied by an inherent feature to support an obviousness rejection. *In re Sernaker*, 217 USPQ 1 (Fed. Cir. 1983).

Claim 1 has been amended to incorporate the recitations of claim 2, and claim 3 depends on claim 1. Claim 2 has been canceled. The skilled artisan would not have been motivated by this combination of references to construct the vector of claims 1 and 3 comprising DNA encoding a heterologous polypeptide, for the following reasons.

As noted above, Hasan does not disclose production of any heterologous polypeptides from its constructed vectors, nor do any of the vectors described contain the GreA- or GreB-encoding DNA as required by claims 1 and 3. The skilled practitioner would have had to look elsewhere for a teaching or suggestion of a vector as claimed, for production of polypeptides heterologous to bacteria.

Hsu explores the parameters that affect promoter escape by *E. coli* RNA polymerase for three different promoters: the phage T7 A1 promoter, the phage T5 N25 promoter, and the chimeric promoter T5 $N25_{antiDSR}$. The activity assay used by Hsu to stimulate RNA chain initiation by Gre factors used the chloramphenicol acetyltransferase (CAT) structural gene (see page 11589, left column, last paragraph before RESULTS). CAT is a bacterial enzyme that detoxifies or inactivates the antibiotic chloramphenicol by acetylation. Since this gene confers chloramphenicol resistance, it is widely used as a marker or selector in genetic transfection experiments, including as a reporter gene for examining the control of eukaryotic gene expression. It is clearly a homologous polypeptide.

Hsu reports on page 11590 (right column) that in the presence of the *greA* plasmid, expression of the homologous CAT protein from a

certain promoter produced an 8-fold CAT activity effect with IPTG induction. However, Hsu points out directly after this that a different promoter, N25, was only weakly affected by GreA overproduction, and GreB supplementation produced essentially the same outcome. Hence, the results in producing the homologous protein were apparently promoter-specific. Furthermore, many mechanistic questions that arose from the findings in Hsu remained unanswered. See p. 11591, left column, first full paragraph, and first sentence of second full paragraph, where many issues presented are not resolved, including whether the Gre factors affect productive initiation. Hsu, therefore, involves mechanistic studies using a CAT activity assay that does **not** produce heterologous polypeptides, in contrast to the vectors claimed herein.

Hsu, representing the prior art literature on GreA/GreB, shows that under some circumstances there is more productive transcription initiation with GreA or GreB, but this would not inform the skilled artisan as to whether translation initiation would also be improved so as to motivate the skilled artisan to construct the vector of claim 1 comprising the DNA encoding the heterologous polypeptide in combination with the other elements. Since there is no explicit suggestion or teaching in these references of the claimed vectors, they are not obvious. There would have been no motivation at the time of the effective filing date to combine the teachings of Hasan on homologous polypeptide production with those of Hsu on an activity assay using a different homologous polypeptide to arrive at the claimed vectors, which utilize DNA encoding heterologous polypeptides.

Hence, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1 and 3 under 35 U.S.C. §103(a) over Hasan in view of Hsu.

Rejection under 35 U.S.C. § 103(a) (Hasan in view of Li *et al.*, Biotechnol. Appl. Biochem., 26: 15-17 (1997)) or Clements *et al.*, Oncogene, 8: 1311-1316 (1993))

Claims 1, 3-4, 10, 12-15, and 17-20 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hasan in view of Li or Clements. According to the Office, it would have been obvious to one of ordinary skill in the art to use the plasmid system of Hasan to produce and purify TPO or FGF-5 as taught by Li or Clements,

respectively, since the purpose of the vector of Hasan was to allow for high-level expression of a protein in bacterial cells, Li and Clements both teach a different protein expressed and purified in bacterial cells, and the skilled artisan always wants high-level expression of the protein to be purified.

First, applicants note that rejected claim 1 now incorporates the recitations of claim 2, which is not rejected in this section. GreA and GreB are nowhere mentioned by the cited references, and thus the amendment to claim 1 moots this rejection of claim 1 and of dependent claims 3-4.

Second, the process claims reflect a system designed specifically to prevent the formation and accumulation of truncated and 10Sa-tagged heterologous proteins, which cause problems with protein purification. One of the primary causes of truncated and 10Sa-tagged proteins is premature transcription termination within a translated coding sequence. The accumulation of full-length protein may be similar with or without the anti-termination system, but the accumulation of the truncated forms is significantly reduced by promoting transcriptional readthrough of intragenic termination signals within the protein's coding sequence.

The minimization of these unwanted truncations of the polypeptide not only allows for simplified purification of the heterologous full-length protein, but minimizing the production of these smaller heterologous fragments also leads to improved efficiency in refolding, since the smaller species may contribute to aggregation in the refolding reactions. In addition, any truncated species that do refold and remain in solution may complicate the assessment of bioactivity by interfering with the binding of full-length protein to the receptor. Thus, it is advantageous to reduce the level of premature transcription termination to prevent these potential problems from arising. See, e.g., page 39, line 38 to page 40, line 7 of the above-identified specification.

Moreover, the general trend in the literature over the years is to eliminate the lambda N anti-termination system that was used with the adjacent P_L promoter system and just use the P_L promoter or replace the N gene with a polylinker or other fusion partner. In contrast, the

invention herein as embodied in claim 6 lies in using the anti-termination system without the P_L promoter.

In keeping with these objectives of the invention, process claim 6, upon which dependent rejected claims 10, 12-15, and 17-20 ultimately depend, has been amended to indicate that it is directed to a process for increasing the percentages of full-length heterologous polypeptide in the overall total heterologous polypeptide mixture obtained. In other words, in process claim 6, applicants are not aiming to increase overall expression of the heterologous polypeptide, but rather the problem they are solving is to clean up truncated polypeptide bands or peaks that appear in assays along with the full-length polypeptide band or peak, so as to obtain more full-length polypeptide on a percentage basis.

Hasan not only does not teach or suggest use of a heterologous polypeptide, but would not instruct or suggest to the skilled practitioner how to solve the problem of ridding a mixture of truncated versions of the polypeptide. Hasan does not even mention the problem of impurities. There would have been no motivation by the skilled artisan to use the plasmid system of Hasan since he used homologous protein and was not aiming to purify in the sense of the claim 6 preamble. Thus, the claimed process would not have been obvious to try at the effective date, let alone be practiced with a reasonable expectation of success. Applicants note that although claim 6 is not rejected in this section, it is being distinguished over the cited art because its recitations are necessarily required in the rejected dependent claims. The other claims necessarily are patentable over the cited art if claim 6 is patentable thereover, since they depend from claim 6.

Li and Clements do not compensate for the deficiencies of Hasan. Neither teaches or suggests how one might obtain their respective proteins as a high percentage of full-length over total protein as set forth in the rejected claims. This claimed result is patentable over these references, as the artisan would not have looked to these references in conjunction with Hasan, since they are non-analogous art.

See, for example, Figure 1 of Li on page 16, wherein the SDS/PAGE analysis of the rhTPO after purification from *E. coli* shows there are still other bands than the full-length rhTPO, with no suggestion or

motivation for how to minimize or eliminate those bands of other rhTPO species. Note page 1312, right column, top, to page 1313, left column, top, of Clements, wherein the authors state that the abundant FGF-5 species migrated as a 29-k-Da protein, which is somewhat larger than expected for unmodified FGF-5 lacking its signal sequence, this being mostly insoluble. The soluble fraction contained lesser amounts of an appropriately sized 27-kDa FGF-5 species. The soluble fraction was used to purify the FGF-5. The largest peak of protein contained, predominantly, a 27-kDa protein along with lesser amounts of a 29-kDa species and smaller proteins. See Fig. 2a and 2b. Fig. 2b shows many bands of these with abundant FGF species migrating.

In contrast, the present invention involves producing the polypeptide with minimal truncations allowing for easy purification and other benefits as noted above. With Li and Clements, further difficult purification steps are needed to eliminate the truncated forms of their proteins (at least for use as a pharmaceutical agent). While mentioning these truncated forms in the Results Section, the authors of Li and Clements essentially avoid their mention after that. Apparently they did not know what caused them and so did not elaborate on them. The claimed invention does not require extra purification steps to accomplish the purpose and results stated in claim 6.

In summary, the skilled artisan would not have turned to any of the cited references alone or in combination to solve the problem of improving percentages of full-length heterologous polypeptide over total heterologous polypeptide as now set forth in claim 6 upon which the rejected process claims depend. Thus, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1, 3-4, 10, 12-15, and 17-20 under 35 U.S.C. §103(a) over Hasan in view of Li or Clements.

Rejection under 35 U.S.C. § 103(a) (Hasan and Li in further view of Makrides, Microbiological Reviews, 60: 512-538 (1996))

Claims 1 and 3-20 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hasan and Li in further view of Makrides. Hasan and Li are discussed above. Makrides is cited for the variety of promoters that can be used in bacteria, and for its teaching of expression of a protein of interest so that it is secreted.

According to the Examiner, it would have been obvious, in the plasmids of Hasan using the expression and purification methods of Li, to use a trp or phoA promoter and express the protein such that it would be secreted into the culture medium as taught by Makrides.

First, claim 1 now recites the GreA or GreB embodiments of claim 2, which is not rejected under this section, so that claims 1 and 3-5 are patentable over this combination of references.

Regarding claims 6-20, Hasan, which deals with a homologous polypeptide, not a heterologous polypeptide, basically is directed to mechanisms and would not be a reference that the skilled practitioner would consult to improve percentages of full-length heterologous polypeptide over total heterologous polypeptide as now set forth in claim 6. Li does not add to Hasan as noted above, as it does not address, let alone suggest, the problem being solved, but merely alludes to the presence of truncated protein species.

Makrides is a review article regarding general strategies for achieving high-level expression of genes in *E. coli*. Just the title alone tells the skilled artisan that this is the review literature to be consulted if one wants to obtain high-level production of recombinant proteins in *E. coli* through the choice of an optimal expression system. It does not direct the practitioner to look for ways to solve the problem as set forth in the present process claims, i.e., to obtain a higher percentage of full-length heterologous polypeptides over truncated versions.

Pages 513-515 discuss promoters that can be used, including Table 1 on page 514 listing various available promoters. This list includes the lambda promoter p_L (λ). On page 514, left column, Makrides states that the most widely used promoters for large-scale protein production use thermal induction (lambda p_L) or chemical inducers (trp). In addition, the phage lambda p_L promoter is discussed in the right column of p. 514 as being a prototype to be studied for cold-responsive promoters. In contrast, the lambda promoter is specifically **excluded** as a promoter from claims 1 and 6 for both the anti-termination nucleic acid and the nucleic acid encoding heterologous polypeptide. (See the definition of non-lambda promoters on page 12, lines 19-23 of the specification, which particularly excludes lambda P_L promoter).

Makrides then describes transcriptional anti-terminators starting on page 515, right column. On p. 515, last line of right column, to page 516, top left column, it is stated that Mertens constructed a reversibly attenuated T7 RNA polymerase expression cassette based on lambda pL regulation. This particular system used the lambda promoter, whereas, in contrast, applicants do not use it in claims 1 and 6, upon which the other rejected claims depend.

Further, nothing in this anti-termination section would indicate to the practitioner that he/she should attempt to use this system for the expressed purpose and result in claim 6. Makrides states on page 515, right column, bottom, that two excellent review articles describe the topic in some detail. Copies of these two review articles (Condon *et al.*, Microbiol. Rev., 59: 623-645 (1995) and Richardson and Greenblatt, in *Escherichia coli and Salmonella*, Cellular and Molecular Biology, eds. Neidhardt *et al.*, Vol. I, second edition (AMS Press, Washington DC 1996), p 822-848) are provided with the IDS accompanying this Amendment for the Examiner's consideration. Most notably, in the last part of this anti-termination section of Makrides, in the last paragraph of this section, on page 516, first column, first full paragraph, the author actually teaches away from the claimed invention. This passage states that:

> The transcriptional antitermination region from the *E. coli rrnB* tRNA operon has been used in the expression vector pSE420, which utilizes the *trc* promoter (64). The rationale in this case was to facilitate transcription through areas of severe secondary structure, thus reducing the possibility of premature transcription termination by the host RNA polymerase. **In this case, however, the presence of the *rrnB* antiterminator is apparently ineffective (64a).** (emphasis added)

This last sentence would have led the ordinarily skilled person not to use the claimed process for expressing, let alone increasing, the percentage of desired polypeptide over undesired in the total production, so as to obtain the heterologous full-length polypeptide as a homogeneous population without species impurities, in the instantly claimed methods and using the instantly claimed vectors. This reference 64a was a personal communication from J. Brosius, who appears to be somewhat of an expert in this area, being cited five times by

Makrides in his review paper (see page 528, first column, references 64-67).

This does not mean, however, that the claimed process is ineffective, since the skilled artisan would have understood from reading the instant specification, including the working examples, how to obtain the claimed result successfully by combining the various elements as appropriate. Further, the present claims at issue specify that the lambda promoter is not employed.

In the section titled Protein Degradation (page 524, right column) Makrides states "Protein damage or alteration may result from a variety of conditions, such as incomplete polypeptides, ... Such abnormal proteins are efficiently removed by the bacterial proteolytic machine." In essence, Makrides again teaches away from the invention by telling readers that the potential problem of accumulating incomplete polypeptides will be taken care of by proteolytic mechanisms.

Finally, Makrides states on page 526, right column, in the Conclusion section, that fusion partners have utility in the production, detection and purification of recombinant proteins (see second full paragraph). The next paragraph mentions solutions to various problems like protein misfolding by use of conditions and partners that have nothing to do with the present invention. Regarding how to deal with protein degradation, in the last full paragraph in that column, the author suggests various solutions that again having nothing to do with anti-termination. The remaining paragraphs on pages 526-527 merely suggest available systems to facilitate secretion and enhance protein yields using such factors or conditions as coexpression of multiple chaperone-encoding genes or activating a large battery of chaperone molecules or using fusion partners. Again, there is no suggestion of the particular problem addressed by instant claim 6, let alone the solution set forth therein.

Since the independent claims are patentable over this combination of references for all the reasons noted above, the dependent claims are also patentable, referring to all the patentable elements of the claims upon which they depend. Hence, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1 and 3-20 under 35 U.S.C. §103(a) over Hasan and Li in view of Makrides.

Rejection under 35 U.S.C. § 103(a) (Hasan and Hsu and further in view of Li)

Claims 1-4, 6-10, 12-15, and 17-22 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hasan and Hsu as applied to claim 2 and further in view of Li. According to the Examiner, the skilled artisan would have been motivated and have found it obvious to combine the teachings of Hasan, Hsu, and Li to express and purify a protein of interest using both the anti-termination system combined with the GreA/GreB system because both the anti-termination system of Hasan and the Gre system of Hsu lead to an increase in production of the protein of interest.

Hasan and Hsu are not relevant to vector claims 1 and 3-4 (claim 2 is canceled) for the reasons as noted above. Li adds nothing to this combination of references that would alter this conclusion, not even mentioning GreA or GreB, let alone how they would be used.

Regarding claims 6-10, 12-15, and 17-22, applicants note that unwanted cleavage of the polypeptide is minimized by inclusion of nucleic acids encoding GreA or GreB. See, e.g., page 6, lines 18-33 of the above-identified application. Hasan and Li do not address the problem solved by the instantly claimed invention, to obtain a higher percentage of full-length heterologous polypeptide over truncated versions. This is fully discussed above. Hsu adds nothing to this discussion, since it is directed to an activity assay for a homologous protein CAT to study the mechanism of promoter escape, and not to producing heterologous polypeptides, let along to solving the problem of the presence of truncated species of polypeptide in a mixture with full-length polypeptide by minimizing production of such species as set forth in claim 6. Hence, all the independent claims being rejected are patentable, and thus also the dependent claims.

For all these reasons, applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-4, 6-10, 12-15, and 17-22 under 35 U.S.C. §103(a) over Hasan and Hsu and further in view of Li.

Rejection under 35 U.S.C. § 103(a) (Li or Clements in view of Hsu)

Claims 23-32 and 34 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Li or Clements in view of Hsu. The

Office contends that Li discloses a vector for the expression of human TPO in bacterial cells and a process for producing and purifying the TPO from inclusion bodies of the bacterial cytoplasm. Similarly, Clements is seen to disclose a vector for the expression of human FGF-5 in bacterial cells and a process for producing and purifying the FGF-5 from the cytoplasm of the bacteria. Hsu is said to disclose a plasmid for expressing GreA or GreB and a process for enhancing the expression of a gene of interest through expression of GreA or GreB (see Figure 3). Accordingly, the Examiner's position is that one would have been motivated to combine the teachings of Li or Clements with those of Hsu, because in production of a protein of interest for the purification of that protein, one desires to have the greatest level of expression of that protein possible. The Examiner states that Hsu teaches that both GreA and GreB can lead to up to an eight-fold induction of expression of a gene of interest (see p. 11590, column 2). Thus, it allegedly would have been obvious to construct and use a vector combining GreA or GreB expression with the gene for expressing the protein of interest.

Vector claims 23-26 have been canceled. Process claim 27, upon which the other rejected claims ultimately depend, now expresses the surprising result, set forth, e.g., on page 6, lines 18-33 of the above specification, of minimizing unwanted cleavage of the polypeptide by including in the vector nucleic acids encoding GreA or GreB. Thus, the problem to be solved by applicants in their claim 27 is not to increase high-level expression of proteins, as the Examiner is contending. The combination of cited references would not have provided the incentive for the skilled artisan to practice the process of claim 27 with a reasonable expectation of success, as it would not have been obvious that the result of maximizing the percentage of full-length polypeptide relative to truncated polypeptide could be achieved. Li and Clements both obtain truncated species, as pointed out above. Figure 1 of Li shows multiple species of hTPO, and Figures 2a and 2b of Clements show abundant FGF species in the peaks and lanes set forth. Neither of these references contain a suggestion for how to minimize the presence of these species, which contaminate the preparation containing full-length polypeptide.

As noted above, Hsu investigates the parameters that affect escape from the promoter by *E. coli* RNA polymerase for three promoters.

The activity assay used by Hsu to explore the mechanism and the stimulation of RNA chain initiation by Gre factors employed experiments using CAT as structural gene (see page 11589, left column, last paragraph before RESULTS). CAT is a bacterial enzyme, so it is not heterologous to the *E. coli* in which it is produced.

The excerpt from Hsu pointed out by the Examiner on page 11590 (right column) states that in the presence of the *greA* plasmid, expression of the homologous protein CAT from a certain promoter produced an 8-fold CAT activity effect with IPTG induction. A different promoter, N25, however, was only weakly affected by GreA overproduction. GreB supplementation produced essentially the same result. See page 11590 (right column). It appears, thus, that the effects on production of the homologous protein were promoter-specific. Moreover, many questions that arose from the results of Hsu's experiments remained unanswered. See p. 11591, left column, first full paragraph, and first sentence of second full paragraph. These unresolved issues include whether the Gre factors affect productive initiation. Hsu represents an exploration of mechanisms regarding the Gre factors using a CAT activity assay that does not produce heterologous polypeptides, as claimed herein. Much less does Hsu, alone or in combination with Li and Clements, teach or suggest minimizing the production of truncated heterologous polypeptide species, which is now a key aim and result of claim 27 upon which the other rejected claims depend.

Since none of the references alone or in combination would have suggested the claimed process features, applicants respectfully request reconsideration and withdrawal of the rejection of claims 23-32 and 34 under 35 U.S.C. §103(a) over Li or Clements in view of Hsu.

Rejection under 35 U.S.C. § 103(a) (Li or Clements and Hsu further in view of Makrides)

Claims 23-35 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Li or Clements and Hsu as applied to claims 23-32 and 34 above, and further in view of Makrides. According to the Examiner, Li or Clements combined with Hsu teaches the limitations of claim 27 as taught above. While Li or Clements and Hsu do not teach the use of trp or phoA promoters and purification from the culture

supernatant, according to the Office, Makrides teaches the use of a *trp* and *phoA* promoter and of methods for and usefulness of protein secretion.

Again, it is pointed out that claims 23-26 have been canceled. As to the remaining claims, claim 27, upon which all the other rejected claims depend, is directed to a method to increase full-length polypeptide as a percentage of total such polypeptide, and not overall expression. None of the cited references address this problem or its solution, nor suggest anything that would be motivating to the skilled artisan to try to solve this problem, let alone attempt to solve it with a reasonable expectation of success. Makrides does not compensate for the deficiencies of Li, Clements, and Hsu in not addressing the specific problem being solved by the instant claims, as noted above. In the Protein Degradation section on page 524 referred to above, Makrides teaches away from the invention of claims 27-35 by stating that abnormal proteins, such as, for example, incomplete polypeptides, are efficiently removed by the bacterial proteolytic machine, rather than by any other means, such as that claimed herein. Additionally, the conclusions in Makrides make no reference to use of GreA or GreB, let alone to their use as a solution to any of the problems of protein production raised therein on pages 526-527 such as purification and refolding difficulties solved by the present invention.

Hence, applicants respectfully request reconsideration and withdrawal of the rejection of claims 27-35 under 35 U.S.C. §103(a) over Li or Clements and Hsu further in view of Makrides.

Summary

In summary, none of the cited prior art authors taught or suggested the vector claims. Further, none even recognized the problem to be solved by the processes in claims 6 and 27, let alone suggested, alone or in combination, the claimed unexpected solutions to minimizing the accumulation of truncated polypeptides of interest. In fact, Makrides taught away from the claimed processes in various respects.

Information Disclosure Statement

The Examiner has requested that applicants resubmit the information disclosure statement (IDS) mailed May 20, 2002. Applicants appreciate the fact that the references have been considered.

Applicants are re-submitting with this Amendment the IDS with darker, readable PTO-1449 forms as requested by the Examiner.

Applicants are also submitting herewith a new IDS (referred to above) for fresh consideration by the Examiner and entry on the record.

If in the opinion of the Examiner, a **telephone conference** would expedite the prosecution of the subject application, the Examiner is **strongly encouraged** to call the undersigned at the number indicated below.

This response/amendment is submitted with a transmittal letter. In the unlikely event that this document is separated from the transmittal letter or if fees are required, applicants petition the Commissioner to authorize charging Deposit Account 07-0630 for any fees required or credits due and any extensions of time necessary to maintain the pendency of this application.

Applicants believe the claims are in condition for allowance and respectfully request a Notice to that effect.

Respectfully submitted,

GENENTECH, INC.

Date: June 22, 2005

By: Janet E. Hasak

Janet E. Hasak
Reg. No. 28,616
Telephone No. (650) 225-1896